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Implication of sludge stabilization process and polymeric material addition on nitrogen and carbon mineralization

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A B S T R A C T

Soil fertility deterioration has been a challenge limiting crop productivity. Recycling municipal sludge in agroecosystems proved to be an effective soil nutrient source. However, due to varied nutrient content emanating from wastewater sources and treatment processes, sludges require application rate optimization for sustainable reuse. A laboratory incubation study was conducted over 90 days to quantify carbon (C) and nitrogen (N) mineralization rate from sludge amended soils. Aerobic (AeD) sludge, anaerobic digested sludges without polymer (AnDP0) and with polymer (AnDP1) treatments were applied at 10 tons ha-1. N fractions and other parameters varied significantly with sludge treatment. AeD had significantly higher total N than AnD sludges. AeD sludge mineralized significantly higher cumulative CO2 – C than AnD. AnD sludges had higher final N mineralization rates of 43% (AnDP0) and 54% (AnDP1) against 41% from AeD sludge. Polymeric material addition increased net N mineralization rate by 10%. Cumulative mineralized N showed to be driven by the size of applied organic N pool. Applied organic N was higher in AeD relative to AnD sludges, leading to higher net N mineralized. N mineralization was faster within first 30 days of sludge application, suggesting that, for efficient mineral N utilization from sludge, planting must be planned to synchronize crop N needs with this high biosolids N release period. The study showed the importance of basing sludge application rates on N content and mineralization rate rather than a single and generalized recommendation rate; a strategy that limits excess nutrient application and reducing pollution whilst enriching agroecosystems.

1. Introduction

Declining soil fertility status in conjunction with high chemical fertilizer costs (Hallama et al., 2019) are repressive to crop productivity. Besides reduction in crop productivity due to water shortages aggravated by climate change (Nyagumbo et al., 2019; Ahmad et al., 2020), soil fertility losses have been reported as major factors limiting crop yields (Romanyà et al., 2017). These challenges, among other factors, have exacerbated the difficulties in fighting hunger and have propelled the state of poverty among the rural communities and smallholder farmers worldwide. Generally, farmers in the developing world are finding it difficult to access mineral fertilizers due to high costs and limited spatial distribution as well as experiencing high frequency of seasonal droughts especially in arid and semi-arid regions (Nyagumbo et al., 2019). Additionally, their landholding is small and do not strongly support multiple cropping and seasonal rotation systems of cereals and leguminous crops to boost soil fertility. Therefore, this has resulted in either practicing monocropping system or larger fraction of their farm land being allocated mainly to staple crops like maize (van Vugt et al., 2018) for most communal farmers. Such cropping systems are not favourable in fixing nitrogen. At the same time, the trade-offs of crop residues between livestock feed and retention for soil fertility build up have shown that most smallholder farmers tend to focus more on livestock feeds relative to soil amendment (Tittonell et al., 2015). Therefore, with such limited crop residue retention, this calls for bringing in other alternative external sources of nutrients to enhance soil fertility and crop productivity.

Municipal wastewater sludge was identified to have substantial amount of required soil nutrients. Its recycling as organic amendment material in agricultural lands could be an alternative option (Jin et al., 2011; Seleiman et al., 2020) to supplement or substitute synthetic fertilizers (Heimersson

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et al., 2017) and rejuvenate degraded soils. However, municipal sludge may have organic and/or inorganic contaminants (da Silva Souza et al., 2020), associated with the source (industrial or domestic origin) (Rouch et al., 2011). Contaminants render and limit sludge usability in agriculture lands as they tend to accumulate and contaminate the ecosystem. Contaminant accumulation could be toxic to the general soil microorganisms which are the "engines" driving the nutrient cycling within soil systems. Some of the contaminants may be taken up by plants hence making their way to the human food chain posing health hazards (Alvarenga et al., 2017).

Sludge that qualifies for agricultural use is usually passed though treatment and stabilization processes (Rouch et al., 2009). Properly treated and stabilized sludge can be a suitable material for recycling, a good source of plant nutrients and be utilized to enrich degraded soils (Černe et al., 2019). However, potential remnants/traces of sludge contaminants could exist even after treatment, therefore not all sludges produced are usable for agricultural production. In addition, due to variation in efficiencies of different treatment and digestion processes employed, sludges would be affected in their organic matter, N content, general chemical composition and characteristics (Černe et al., 2019). Such variation in sludge characteristics affects the amount and release characteristics of the much-needed plant nutrients like N when applied in the land.

Sludge is usualy applied based on crop N and/or P requirements as guiding principles for sustainable agroecosystems management (Li et al., 2012; Rigby et al., 2016). This is, alternatively, regarded as agronomic application rate (Vieira et al., 2005) which is normally set by regulatory authorities responsible for developing sludge use guidelines (Snyman and Herselman, 2006; C. US EPA, 1993). Guidelines are set such that only sludges that meet certain contaminants threshold limits would be applied at certain application rates that are commensurate with required nutrients to reduce pollutants accumulation and nutrient enrichment. Excess application of N and P may leads to surface water body contamination (eutrophication) (Schoumans et al., 2014; van der Bom et al., 2019) through erosion and runoff and ground water contamination (nitrate leaching) (Manirakiza et al., 2019). Therefore, sludge land application according to crop nutrient requirement is key for sustainable sludge utilization. However, a large proportion (>80%) of the N in sludge is in organic form (Vieira et al., 2005) whilst crops require and take up inorganic N. Determining N release rate of sludges is crucial in order to understand the potential amount of inorganic N sludge application would contribute (Rigby et al., 2016).

Mineralization is the transformation of organic N into inorganic N through the intermediary of microbes (Bruun et al., 2006). As a biological process, N mineralization is influenced by abiotic factors such as climate, soil characteristics and organic matter composition (sludge properties) (Cogger et al., 2004). The intrinsic properties that affect sludge N mineralization rate are born out of effluent composition, treatment and post-treatment drying and handling processes (Cogger et al., 2004; Rigby et al., 2010). It is therefore of utmost importance to understand and quantify the effect of sludge treatement and post-treatment handling processes on the N release characteristics of sludge for sustainable sludge use in agricultural lands.

Many mineralization studies have been carried out, involving various sludges or other organic amendments (Li et al., 2012; Azeez and Van Averbeke, 2010; Rehman and Qayyum, 2019). In their work Hseu and Huang (2005) investigated N mineralization from soils amended with biosolids; over a period of 48 weeks they recorded a range of mineralization rate between 3 and 34% of the total N applied. The findings showed that rate of mineralization was based among other factors on applied N. Whilst in another study, Rigby et al. (2016) revealed a wide variation in mineralization rate which was attributed mainly to sludge type and their associated treatment process. In addition, the observed variations were due to magnitude of their nutrient content and bioavailability (Seleiman et al., 2020; Dad et al., 2019). As well, with several factors including source/origin, that is, industrial or domestic, (of which the latter may be due to households' diets) and treatment processes among other factors (Seleiman et al., 2020; Ghirardini and Verlicchi, 2019), large variations of N release outcomes should be expected on different studies. Such outcome variations strongly warrant each organic amendment be chemically evaluated to determine its specific mineralization rate under specific conditions when it is recycled in agricultural lands. This would allow the organic materials to be utilized in a sustainable way (Sharma et al., 2017). With such confounding findings being observed across studies, it becomes difficult to make reliable conclusions, therefore, this justifies the importance of having separate studies based on each sludge produced from its own origin even it could have gone through presumably similar treatment process. This research therefore is building on the existing international knowledge and is a foundation at the local scale on N release from different treated sludges originating from the same source and help in guiding sustainable application rates that would limit excess application of nutrients into agroecosystems and curtail environmental contamination.

This study is aimed at quantifying the potential N mineralization and C decomposition rates from soils amended with three different sludge types through laboratory incubation studies. We hypothesized that sludge stabilization processes and polymer addition would significantly influence N and C mineralization rates of sludge.

2. Materials and methods

2.1. Soil

A sandy loam soil was used for the current laboratory incubation study. The soil was collected from the University of Pretoria's experimental farm and is classified as Hutton soil which are loamy, kaolinitic, mesic, Typic Eutrustox (Soil Classification Working Group, 1991).

2.2. Sludge types used

Three different candidate sludges were selected for this study. The sludges were all collected from a single wastewater treatment plant (WWTP) at which they underwent different levels of treatment. It was the different levels of treatment that differentiated the three sludge types for the study. The candidate sludges were aerobically digested (collected before the anaerobic digester) (AeD), anaerobically digested sludge without polymer addition (AnDP0), and anaerobically digested sludge with polymer (AnDP1). However, they underwent a similar dewatering/drying process in sand drying beds. All sludges were dried at 25 cm drying thickness in drying beds until they reached 90% solid concentration (10% moisture content). To attain the intended moisture content, it took these sludges a drying period of 45 days from August to September season in 2017.

The anaerobically digested sludge was produced through conventional mesophilic anaerobic process. This process produces a well stabilized product. At the time of collection and beginning of drying, the sludge had ±97% water content. The normal anaerobic sludge (without polymer) for the study, was collected just before the sludge – polymer mixing point and sent to the drying beds. To enhance the dewatering process, the WWTP that supplied the sludge adds chemical conditioning that improves flocculation of the product solids and separates them from excess water. Whilst WWTPs use varied chemicals for conditioning, the current WWTP largely treats its sludge with cationic polymeric material and therefore, for the candidate sludge that needed addition of polymer for the study was treated with the same material. This sludge (AnDP1) was treated with FLOPAM™ FO 4490 which is an organic cationic polymeric flocculant. Ideally, this polymeric material when added to sludge easies the surface tension of liquid-to-liquid and liquid-to-solids interphases and enhances compressibility and dewatering processes especially by WWTPs that do belt pressing than drying in beds. However, for the polymer treatment for this current study, the sludge was not passed through the belt-press, rather it by-passed the belt-press, and was then treated with polymer, from which it was taken directly to the drying beds.

In brief, polymers exist as organic or inorganic materials which are either cationic, non-ionic, amphoteric or anionic charged materials (Lee et al., 2014). Their major purpose is to enhancing effluent flocculation which influences dewaterability ideally for WWTPs that use belt pressing as their sludge dewatering technique (Jiang and Zhu, 2014). Flocculation is a process involving aggregation of suspended particles through bridging or patch mechanism (Sharma et al., 2006). This process is seen as a simple way of separating solid-liquid fractions during which suspended and dissolved solids, colloids and organic matter are efficiently removed from wastewaters (Renault et al., 2009). Generally, the working principle of these polymers is based on their charged sites in which upon application to wastewater, they get attracted, attached to the negatively charged surfaces of the colloids through which they neutralize these negatively charged particles and bridge the destabilized particles together forming some flocs (Chong, 2012; Suopajärvi et al., 2013).

The WWTP from which the sludges were collected mainly treats its sludge through anaerobic digestion. However, during periods of high influent inflow, and the WWTP is under immense pressure with no free anaerobic digesters to take up all aerobic sludges, the plant would then divert and channel aerobic sludge to the paddies to free the treatment system. Therefore, aerobic sludge (AeD) for this study was collected from the same WWTP like AnD but just before it is passed through to anaerobic digestion process. Aerobic digestion involves oxidation process that supplies oxygen to the microbes responsible for digestion. The amount of oxygen required is based on the amount of volatile solids to be oxidized. Volatile solids that are stabilized and converted into gases during aerobic digestion process are not likely to exceed 50%, hence making this sludge type less stable than AnD and very low in its solids content. Due to that effect, proper dewatering of aerobic sludge is not as easy and affordable as other sludge types. Therefore, in most instances at the current WWTP, AeD sludge would be channelled to paddies to free the treatment system. The candidate sludge used for this study was therefore collected from secondary clarifiers as liquid sludge of ±97% moisture content.

2.3. Laboratory incubation settings

N and C mineralization from three candidate sludges used in this study was estimated based on a laboratory incubation experiment that was conducted at the University of Pretoria over a period of 90 days.

2.3.1. N mineralization

The incubation study to estimate N mineralization was conducted in airtight one litre white plastic containers. A soil alone (control) and soil – sludge mix (treatment) were prepared. The treatments were made in three replications. The containers were placed and kept in a dark incubation chamber maintained at constant temperature of 25 ± 1 °C. A soil – sludge mix was prepared of a 0.5 g sludge applied to 100 g soil and mixed thoroughly. The application rate of 0.5 g sludge to 100 g soil was based on the recommended sludge application rate of 10 tons per hectare as stipulated in the South African sludge use guideline (Snyman and Herselman, 2006) assuming a soil bulk density of 1300 kg m^{-3} and incorporation depth of 0.15 m. Upon mixing the soil and sludge thoroughly, deionised water was applied such that the soil moisture in the incubation containers was maintained at field capacity. Throughout the incubation period, soil moisture was maintained at field capacity based on gravimetric water content differences. An average of five treatment containers were randomly selected and weighed to determine the mass difference and deionised water would be applied if need be. To ensure the treatment system was kept under aerobic conditions, containers were opened for oxidation once every week for 3–5 min. Sampling during incubation was done at day 0, 30 and 90 after sludge application.

Sludge N mineralization rate was then estimated using the method as described by Azeez and Van Averbeke (2010) and Rouch et al. (2011). At any sampling time, Mineral N/inorganic N was calculated as the difference in mineral N released between soil–sludge mix treatment (amended soil) and control (unamended soil) (Hanselman et al., 2004); that is:

$$\text{Mineral N}_{(t=0;\,x)} = \text{Mineral N}_{(\text{amended }(t=0;\,x))} - \text{Mineral N}_{(\text{control }(t=0;\,x))}$$

$$\text{Net N mineralization rate (NMR) (\%)} = \left[\text{Total mineralized N from sludge}_{(t=x)} / \text{Organic N }\left(_{\text{applied}}\right)_{(t=0)}\right] \times 100$$

where;

$$\text{Organic N }\left(_{\text{applied}}\right) = \left[\text{Total N}_{(t=0)} - \text{Mineral N}_{(t=0)}\right]_{(\text{amended})} - \left[\text{Total N}_{(t=0)} - \text{Mineral N}_{(t=0)}\right]_{(\text{control})}$$

$$\text{Mineral N} = NH_4\text{–N} + NO_3\text{–N}$$

$t = 0$ means initial or Day 0 (day of starting the experiment); $t = x$ is Day 30 or Day 90 (or at any sampling day after Day 0).

2.3.2. Carbon dioxide evolution

The carbon decomposition and CO_2 evolution study was conducted in airtight desiccators and the experiment ran concurrently with N mineralization study under the same environmental conditions. A soil – sludge mix was prepared in the same manner as for the N mineralization study where 100 g soil was mixed with 0.5 g of sludge and included another set of soil alone (control). Sludge amended soil and soil alone treatments were incubated in desiccators along with a beaker containing an aqueous solution of 20 ml sodium hydroxide (NaOH) to trap the evolved CO_2 and would be replaced on each sampling day. To keep the treatments moist during the incubation period, a beaker with 20 ml of deionised water was put in each desiccator alongside the NaOH solution. The desiccators were sealed and incubated in the same incubation chambers as described above. The sampling time and aeration time of the treatments were done at day 1, 3, 7, 15, 30, 60 and 90. To determine the evolved CO_2 per given time period, the alkali (NaOH) solution that could have reacted chemically with CO_2 during the incubation period was mixed with 4 ml barium

Table 1
Physicochemical characteristics of the soil and sludge types (aerobic (AeD) and anaerobic without polymer (AnDP0) and anaerobic with polymer (AnDP1)) used for the incubation study.

Parameters	Units	Soil	AeD	AnDP0	AnDP1
PH (H_2O)	–	5.9	5.8 a[1]	6.4 b	6.4 b
EC	mS m^{-1}	4	243 a	937 b	1031 b
Total N	%	0.05	6.9 b	4.4 a	4.2 a
NH4 – N	g kg^{-1}	<1	2.3 a	8.1 b	7.2 b
NO3 – N	mg kg^{-1}	3.6	8.6 b	4.7 a	9.2 b
Total C	%	0.5	38 c	34 b	32 a
Organic C	%	0.4	26 c	24 a	25 b
OM	%	0.6	45 c	41 a	42 b
C:N	–	10.8	5.3 a	7.2 b	7.9 c
P	g kg^{-1}	0.6	24 c	20 b	19 a
Extractable P	mg kg^{-1}	49	82 a	119 b	110 b
K	mg kg^{-1}	179	3868	1825	1562
Ca	mg kg^{-1}	706	16,602	24,920	23,252
Mg	mg kg^{-1}	276	5773	5299	4289
Mn	mg kg^{-1}	326	487	763	807
Na	mg kg^{-1}	107	1697	1545	1355
Al	mg kg^{-1}	8720	7471	11,821	11,264
Zn	mg kg^{-1}	29	1034	1695	1600
Fe	mg kg^{-1}	33,635	13,761	16,631	16,152
Cu	mg kg^{-1}	17	159	242	228
B	mg kg^{-1}	96	76	70	70
Sand	%	72	–	–	–
Silt	%	11	–	–	–
Clay	%	18	–	–	–

[1] Means followed by different letters across sludge types are significantly different at $\alpha = 0.05$.

chloride ($BaCl_2$). A 2 ml of Phenolphthalein, an indicator solution, was added to the solution and 0.5 *M* Hydrochloric acid (HCl) was used for titration in relation to the amount of trapped CO_2. The quantity of HCl (ml) used for titrating was then used to compute the CO_2 flux for each sampling day as described by Zibilske (Zibilske, 1994).

$$CO_2 \text{ flux } \left(\text{mg kg}^{-1} \text{ soil}\right) = [(\text{B–V}) \times \text{NE}]/\text{m}$$

where;
B = volume (ml) of 0.5 M HCl needed to titrate the NaOH for the control,
V = volume (ml of 0.5 M HCl needed to titrate the NaOH for the amended sample,
N = molarity of the HCl (0.5 M),
E = equivalent weight; to express as milligrams of CO_2 – C (6)
m = mass of soil (kg)

2.4. Physical and chemical analysis

A fraction of the soil and sludge samples used for the study were sent to Agricultural Research Council (ARC) - Institute for Soil Climate and Water laboratory for analysis of total N, total C and inorganic N. All other elemental analyses, pH, EC and organic C and selected physical properties of the soil were analysed at the Soil Science Laboratory of the University of Pretoria. The chemical characteristics of all the materials used are shown in Table 1.

Soil textural analysis was conducted using hydrometer method following OM removal by hydrogen peroxide oxidation. The soil, sludge and soil-sludge mix electrical conductivity (EC) and pH were measured in a soil or sludge – water suspension extracted from a saturated paste (1,2.5 sample to water ratio). EC was measured using an EC meter (Consort C861) and pH using a glass electrode pH meter (Consort C830), multi – parameter analyzer, Sep Sci, Belgium. Samples ground to pass through 2 mm sieve were extracted using KCl at a ratio of 1 g:10 ml (sample: KCl) for ammonium and nitrate N analyses. These were analysed using colorimetric method with Lachat Auto-analyzer (Lacht Quick Chem Systems, Milwaukee, MI) USA. Whilst the NO_3^- – N, and NO_2^- – N were analysed using Ion Chromatography. Total carbon (TC) and total nitrogen (TN) were analysed by total combustion method using a Carlo Erba Na1500 C:N:S analyzer (Carlo Erba Strumentazione, Millan, Italy). Inductively Coupled Plasma – Optical Emission Spectrometer (ICP - OES) was used for total elemental analyses of Ca, Na, Mg, Mn, S, Fe, Al, K, Cu, Zn, P and heavy metals (in biosolids) (Hg; Cd; Cr; V; Pb; Ni and As) after microwave-assisted nitric acid – perchloric acid mixture digestion. Extractable P was determined using Mehlich III for soil and P-Bray 1 test for sludge samples. Soil and sludge organic carbon and organic matter (OM) were determined after wet oxidation method by Walkley and Black (Walkley and Black, 1934).

2.5. Statistical analysis

SigmaPlot 13.0 version statistical package was used to test the treatment effects on N mineralization rate and other parameters analysed. The data was subjected to Analysis of Variance (ANOVA) at a threshold *P* value of 0.05 with sludge types and sampling days as main factors. Where treatment effects were significantly different, the Duncan Multiple Range test (α = 0.05) was used to separate the means.

3. Results and discussion

3.1. Soil and sludges properties

The general soil characteristics are presented on Table 1. The soil pH of 5.9 (H_2O), was within the range suitable for microbes mediated processes like mineralization and OM decomposition to occur normally. The soil's N content was very low as its TN was below 0.1%, with <1 g NH_4 – N and < 4 mg NO_3 – N kg^{-1} (Table 1), hence warranting nutrient supplements from external sources to boost its fertility status.

The total P and extractable P (Mehlich III) were 0.6 g and 49 mg kg^{-1} respectively. The soil total C content was 0.5%, as a result, its C:N ratio (11) was comparatively higher than that of the sludges used for the study. According to Persson and Kirchmann (Persson and Kirchmann, 1994), soils with C: N ratio within the ranges reported in the current study, harbour favourable conditions to influence OM decomposition process. C:N ratio generally influences microbial activities which in turn affect nitrogen release and its availability for plant uptake in agricultural lands (Rigby et al., 2016; Yang et al., 2020).

However, higher C:N implies that this soil type would need more N supply than what is currently contained in the soil to sustain microbial activities since net mineralization tends to decrease when soil C:N ratio increases (Colman and Schimel, 2013).

An array of selected chemical characteristics of sludges used in this study significantly differed with sludge type (Table 2). The current analyses indicate a wide variation in the magnitude of nutrient quality with sludge type and treatment process. AeD sludge had significantly (P < 0.05) higher TN and TP relative to AnD sludges (Table 1). Rigby, Clarke (Rigby et al., 2016) gave a detailed account of how different sludge treatment processes affect sludge N content and its mineralization. However, between AnD sludges, N was not significantly (P > 0.05) different. Nitrogen and P nutrients have also been reported to be higher in aerobic than anaerobic stabilized sludge (Černe et al., 2019) and our findings are in agreement with this observation. Under such cases, it is therefore crucial to have cautious nutrient management strategies such as observing sustainable application rates when applying organic materials like AeD to minimize oversupply of N and or P. Potential excess application of such nutrients is possibly higher when applying sludges as those in the current study.

Extractable P concentration for AnD sludges did not show significant difference (P > 0.05) between the one with polymer and without polymer although their TP differed significantly (P < 0.05). Total C differed significantly (P < 0.05) among sludge types, however, for organic C and organic matter significant difference was between AeD and the two AnD sludges of which the latter were not statistically different (Table 1). AeD sludge recorded higher percentages in all C fractions and OM over AnD sludges. NH_4 – N was significantly lower in AeD compared to AnD sludges of which no difference was observed between the latter, whilst for NO_3 – N, AeD and AnDP1 were not different and were twice higher in concentration than AnDP0.

The three sludge types had their inorganic N dominated by NH_4 – N compared to NO_3 – N. This is in agreement with previous findings by Rigby, Clarke (Rigby et al., 2016) who reported higher NH_4 – N relative to NO_3 – N in most municipal sludges.

Sludge type significantly affected C:N (Table 2) and it differed statistically (P < 0.05) across the three sludges (Table 1). Comparatively, higher C:N ratio was observed in the two AnD sludges relative to AeD. Organic materials' C:N ratio is an important parameter influencing microbial activities and net N mineralization (Tambone and Adani, 2017). C:N is one of the primary predictors of material stability (Černe et al., 2019). Organic materials

Table 2
Analysis of variance for the measured parameters during the study as influenced by sludge type.

	PH	EC	C:N	TN	NH_4 – N	NO_3 – N	TP	Extractable P	TC	Organic C	Organic C
Sludge type	***	***	***	***	***	***	***	***	***	ns	ns

*, ** and *** indicate significance at P < 0.05, P < 0.01 and P < 0.001 respectively. ns indicates no significant difference at P = 0.05.

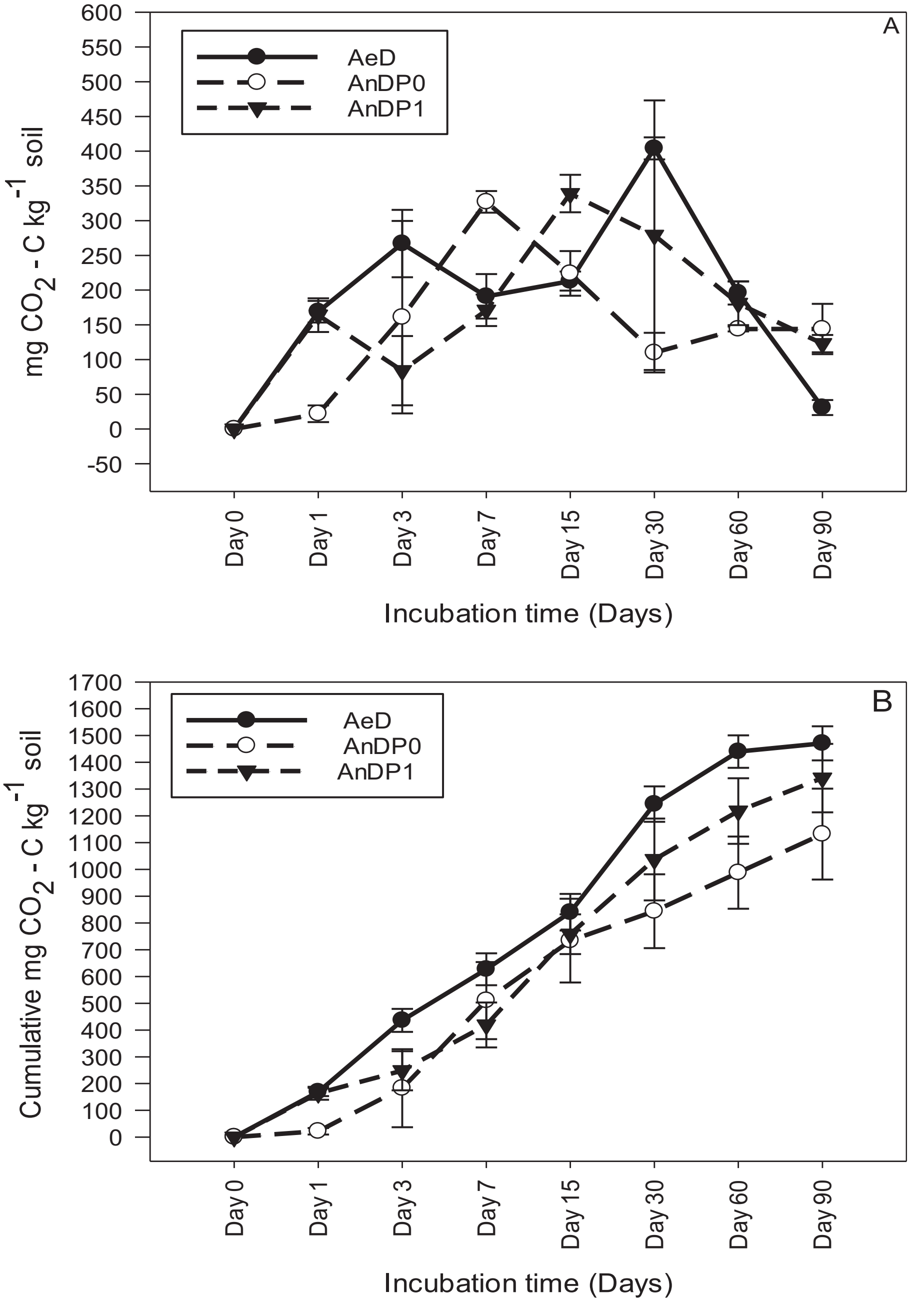

Fig. 1. Time series (A) and cumulative (B) mg $CO_2 - C\ kg^{-1}$ soil evolved from aerobic (AeD) and anaerobic without polymer (AnDP0) and anaerobic with polymer (AnDP1) sludges during incubation period. Error bars represent standard deviation from the mean of three replications per treatment at each sampling day.

with higher C:N ratio normally tend to reduce inorganic N release (Azeez and Van Averbeke, 2010) due to net N immobilization. This implies a negative N period especially in the earlier days of application due to fixation of available and early transformed mineral N by microbes for their own energy (Janssen, 1996). Such a scenario is quite opposite with those materials with low C:N ratio (Rigby et al., 2016). As such, AeD sludge in this case would be expected to decompose rapidly during incubation resulting in early and faster release of mineral N due to, among other factors, its low C:N ratio compared to AnD sludges (Lynch et al., 2016).

Electrical conductivity values varied significantly ($P < 0.05$) among sludge types (Table 1). Highest EC was observed in AnDP1 followed by AnDP0 with AeD recording the lowest EC of 243 $mS\ m^{-1}$. Higher EC in AnD sludges shows the effect of treatment processes. During treatment, salts are added as chemical P removal processes for anaerobic treatment of which these would in turn increase the EC of the resultant sludge. EC is another factor that may influence N mineralization as it negatively affects microbial activities responsible for driving OM decomposition and mineralization process when contents of salts are high (Irshad et al., 2005). Other macro and micro-nutrients analysed varied across the sludges.

3.2. Carbon dioxide evolution

Carbon dioxide evolution or C mineralization is reported to be a predictor of soil health (Castro Bustamante and Hartz, 2016) and an indicator of soil microbial activity (Awale et al., 2017). Its magnitude upon organic amendment is related to organic C stability (Zhao et al., 2008). Figs.1A and B present dynamics of C from three sludge types during an incubation study. AeD sludge showed a sharp increase in $CO_2 - C$ release in the first three days as did the AnDP0 from day 1 to day 7 (Fig. 1A). AnDP1 had its sharp rise in C mineralization from day 3 to day 15 (Fig. 1A). The analysis done showed significant ($P = 0.032$) difference between sludge types and this was mostly observed from Day 1 to Day 30. In most cases, the significant differences were observed between AeD and AnDP0 sludges. Significant ($P < 0.05$) difference in mineralized C was also observed at day 1, 7, 15 and 30 between AnD sludges. Mineralization rate started to decrease at different magnitudes along the incubation period with AnDP0 steadily decreasing from day 7, and AnDP1 from day 15. For AeD sludge, a sharp drop was observed from day 30 until the end of incubation period with a final reading of 31 mg $CO_2 - C$ kg^{-1} soil, which was significantly lower ($P < 0.028$) than the $CO_2 - C$ measure from AnDP0. Studies have shown that there is a positive correlation between C mineralization and CO_2 emission into soil and atmosphere, in which low magnitude of mineralization implies low CO_2 emission and vice-versa (Abdelhafez et al., 2018). As such, measuring this process is critical as it reveals broader impact on management, climate change, and soil nutrient cycling assessment (Haney et al., 2012).

Although variations in C mineralization existed along the incubation period, all sludges maintained a steady increase in their cumulative $CO_2 - C$ release as time progressed until day 90 (Fig. 1B). $CO_2 - C$ evolution from AeD was greater than from the AnD sludges during the first 60 days of the incubation.

However, AeD started levelling off in the last 30 days of the study period, whilst for AnD sludges, $CO_2 - C$ mineralized continued to increase until day 90. At all sampling days, AeD remained higher in its $CO_2 - C$ evolution than AnD sludges from day 1 to the end. By day 90, the cumulative $CO_2 - C$ evolution totalled to 1471, 1132 and 1341 mg kg^{-1} soil for AeD, AnDP0, AnDP1 sludges, respectively. Of the total mg $CO_2 - C$ kg^{-1} soil mineralized, only AeD and AnDP0 release were significantly ($P = 0.020$) different. Although addition of cationic polymer material (AnDP1) appeared to influence C mineralization, its effect was not statistically significant compared with the sludge which was not treated with polymer (AnDP0). Generally, C mineralization and the release of $CO_2 - C$ are processes influenced by microbial actions, material composition and its quality (Hossain et al., 2017). Larger mineralized C observed in AeD compared to AnD indicates the differences in the presence of organic compounds that can be easily degraded (Fernández et al., 2007) in AeD compared with AnD. This is likely so because AeD is not a well stabilized material and has much of its OM going through maturation process during incubation which is quite contrary to materials like AnD sludge that are well stabilized from the treatment plant by time of application. In addition, soil microbes like nitrifying bacteria (Grzyb et al., 2020) work well with increased microbial activities on materials of low C:N ratio and high N content (Dridi and Gueddari, 2019; Lazicki et al., 2020), which is true for AeD (C:N ratio of 5.2) compared with AnD (C:N ratio of >7). In this case, application of AnD sludge is likely to have reduced CO_2 emission into the soil and atmosphere relative to soils treated with AeD sludges. Apparently, under such instances, at field level, application of sludge materials as AnD may enhance C sequestration hence curtailing green-house gases emission and mitigate climate change in the long run.

3.3. Organic N applied from sludge

The organic N concentration applied from each sludge type is presented on Fig. 2. Organic N applied from individual sludge was computed as the difference between TN and inorganic N and less their corresponding control readings observed in the initial (day 0) samples concentrations. Organic N forms an integral part of TN in the soil system. It is from this organic N fraction that inorganic N is mineralized through soil microbial activities and made available for plant uptake. Municipal sludge contains predominantly organic N compared to its mineral N (Rigby et al., 2016; Cogger et al., 2004).

Candidate sludges used in this current study had their organic N fractions constituting about 94% of the applied total N for AeD, 88% (AnDP0) and 85% for AnDP1. AeD had almost two times higher organic N than the AnD sludges (Fig. 2). The observed differences of about ±9% of organic N fraction between AeD and AnD sludges can be attributed to the degree of stabilization the sludges received during treatment process. This is an important N fraction that if not well managed in agricultural lands would result in N leaching and contamination of the agroecosystems (Rigby and Smith, 2013) since its content and source drive N dynamics in soils. Unstabilized organic materials tend to have higher organic N due to

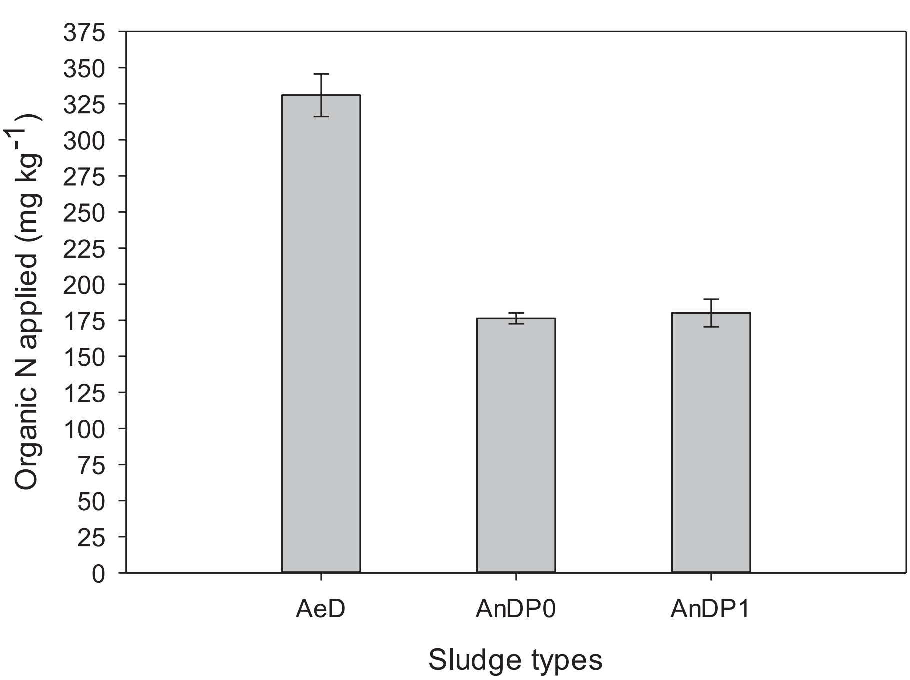

Fig. 2. Organic N applied from aerobic (AeD) and anaerobic without polymer (AnDP0) and anaerobic with polymer (AnDP1) sludges. Error bars represent standard deviation from the mean of three replications per treatment.

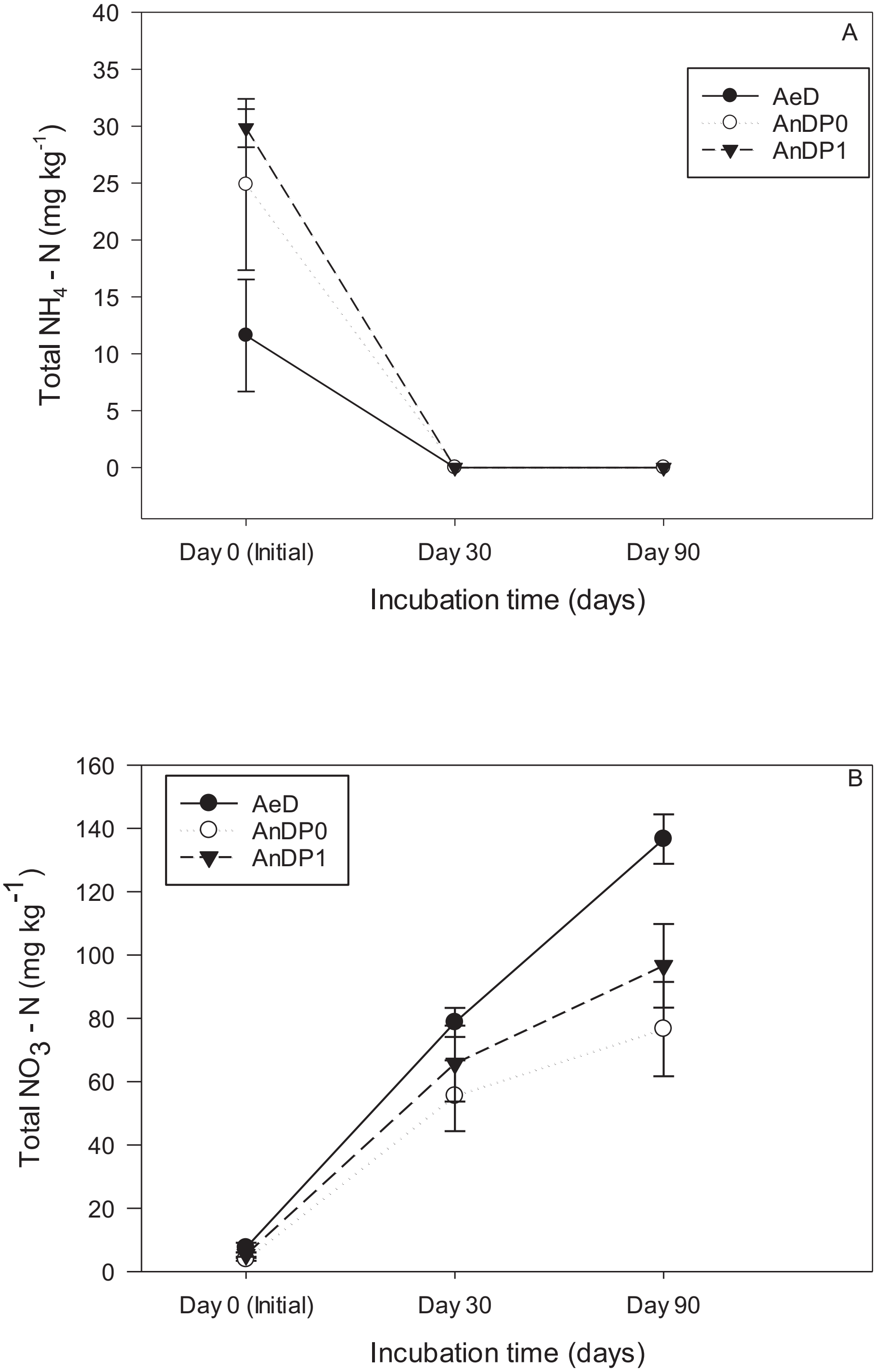

Fig. 3. Total ammonification (A) and nitrification (B) observed from aerobic (AeD) and anaerobic without polymer (AnDP0) and anaerobic with polymer (AnDP1) sludges. Error bars represent standard deviation from the mean of three replications per treatment.

high OM that could still be decomposed, and subsequently release more mineral N compared to well stabilized materials.

3.4. Ammonification and nitrification during incubation

Ammonification and nitrification are the two microbiologically mediated nitrogen transformation processes (Janssen, 1996). The two processes are mediated by heterotrophic microorganisms and utilise organic N (He et al., 2003) and autotrophic nitrifying bacteria that use ammonia and nitrite (Prosser, 1990) as their source of energy and growth. These processes are basically an indication of applied organic N transformation into inorganic N which includes NH_4 – N (ammonification) and NO_3 – N (nitrification). Ammonification during the incubation period was assessed (Fig. 3A). Initially, AnD sludges had more than twice higher NH_4 – N content relative to AeD. This is likely due to the degree of stabilization done to these sludges at the WWTP (Badza et al., 2020a) during which NH_4 – N could be released from digestion of N-rich organic materials (Yang et al., 2018). There was a downward trend (decrease from the initial) in ammonification for the three sludges from day 1 of incubation, and all these sludges had no measurable NH_4 – N at days 30 and 90 (Fig. 3A).

For all sludges in the study, low net ammonification was maintained throughout the incubation period. Ammonification remained at stable

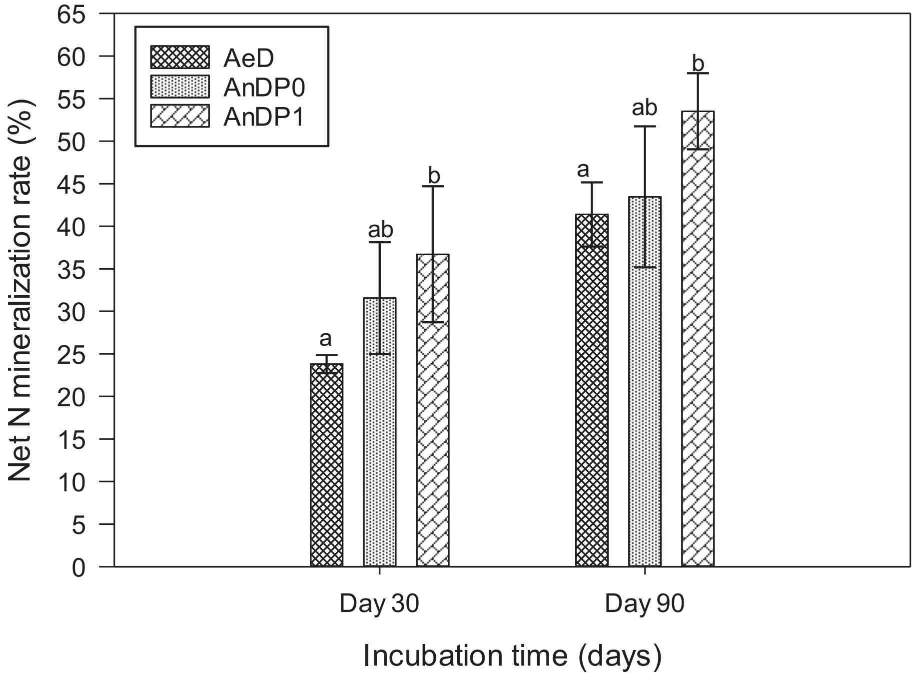

Fig. 4. Net N mineralization rate (%) from aerobic (AeD) and anaerobic without polymer (AnDP0) and anaerobic with polymer (AnDP1) sludges during the incubation period. Error bars represent standard deviation from the mean of three replications per treatment. Different letters on bars per each incubation time represent statistical difference between the means at $\alpha = 0.05$.

state as from Day 30 until the end of the study. This, however, does not mean ammonification was not occurring, rather it is an indication that all ammonium produced was quickly nitrified. Therefore, the observed stable ammonification maintained at zero level (between day 30 to 90) was due to $NH_4 - N$ being transformed into $NO_3 - N$ through nitrification (Fan et al., 2015) or taken up by soil microbes for their use (Dridi and Gueddari, 2019) so rapidly that no $NH_4 - N$ build up could be visible.

Contrary to ammonification trends, nitrification showed a positive trend (Fig. 3B). Initially (Day 0), AnD sludges had 4.7 and 9.2 mg kg^{-1} $NO_3 - N$ for AnDP0 and AnDP1 respectively whilst AeD recorded 8.6 mg kg^{-1}. From the day one of incubation, $NO_3 - N$ increased rapidly in addition to what was applied initially. The progressive increase in $NO_3 - N$ would be attributed to nitrification which took place during the incubation period, which could also be reflected by the observed decrease in $NH_4 - N$ content from both the initially observed at day zero and the $NH_4 - N$ mineralized during the incubation period. However, $NO_3 - N$ content was clearly not entirely from the initially observed $NH_4 - N$ content due to the differences in concentrations of the two N fractions per given time. Most of the nitrate accumulation is likely from mineralization of organic N and then rapid nitrification of the $NH_4 - N$ produced by mineralization.

3.5. Net N mineralization from sludges

Nitrogen mineralization is a biological process that involves soil microbial activities in conjunction with the influence of other factors within the soil systems during organic matter turnover (Abdelhafez et al., 2018). This process happens largely when mineral N in the soil amended with organic material exceeds the N requirements for the soil microbes (Rigby et al., 2016), otherwise the resultant effect could be N immobilization until enough mineral N is accumulated above the microorganisms' requirements. Net N mineralization rate (NMR%) from soil amended with the three sludges over a period of 30 and 90 days of incubation is presented in Fig. 4.

Net N mineralization rate recorded at day 90 represents the overall rate for the whole study period from the initial day of incubation. N mineralization varied significantly ($P < 0.05$) with sludge type. Both AnD sludges showed higher net mineralization rate relative to AeD sludge at Day 90 (Fig. 4) with AnDP1 showing significantly ($P < 0.05$) higher net N mineralization over AeD sludge on the two both on Day 30 and 90.

Although there was no statistical difference on N mineralization rate between the AnD sludges, there was a trend of increased mineralization with the polymer. About 10% mean N mineralization rate differences between AnD sludges was noticed at both Day 30 and 90. The polymer materials affect the physical properties of the sludge during dewatering processes (Badza et al., 2020b). However, the mechanism of any possible polymer effect on biological process such as N or C mineralization under the conditions of the incubations is unclear.

It is evident that mineralization is higher and faster in the early days of organic amendment application. In this study, a higher degree of N mineralization was observed in the first 30 days relative to the last segment (60 days) of incubation (Fig. 4). AeD had 41.4% N mineralization rate by the end of the study but only a 17.6% increase from the observed mineralization rate at Day 30. Whilst, for sludges that underwent through anaerobic digestion, about 12% (AnDP0) and 16% (AnDP1) increase were observed from Day 30 to Day 90.

Nitrogen mineralization rates by the end of the study ranged from 41% for AeD sludges to 43–54% for AnD sludges. Both treatment process and polymer addition may have affected N mineralization, although overall differences in mineralization rates are small. Also, the N mineralization rate of the AeD sludge was similar to or less than the rates for the AnD sludges (Fig. 4), contrary to expected results based on the lower C:N ratio and stability of the AeD material. It is evident that, although C:N ratio is a key factor largely used to predict OM mineralization (Tambone and Adani, 2017), the process does not completely rely on this factor alone, there could be other possible intrinsic characteristics of organic materials' compounds that are critical and likely to drive differences in N release (Tambone and Adani, 2017). Previous authors posit that organic materials with closely related C:N ratio may significantly release variable N quantities during mineralization (Alburquerque et al., 2012; Šimon et al., 2015). The sludges in this study were air-dried in sand beds, and the drying process may have increased the stability and reduced C and N mineralization rates of the AeD sludge (Rigby et al., 2016). Previously, several studies observed varied

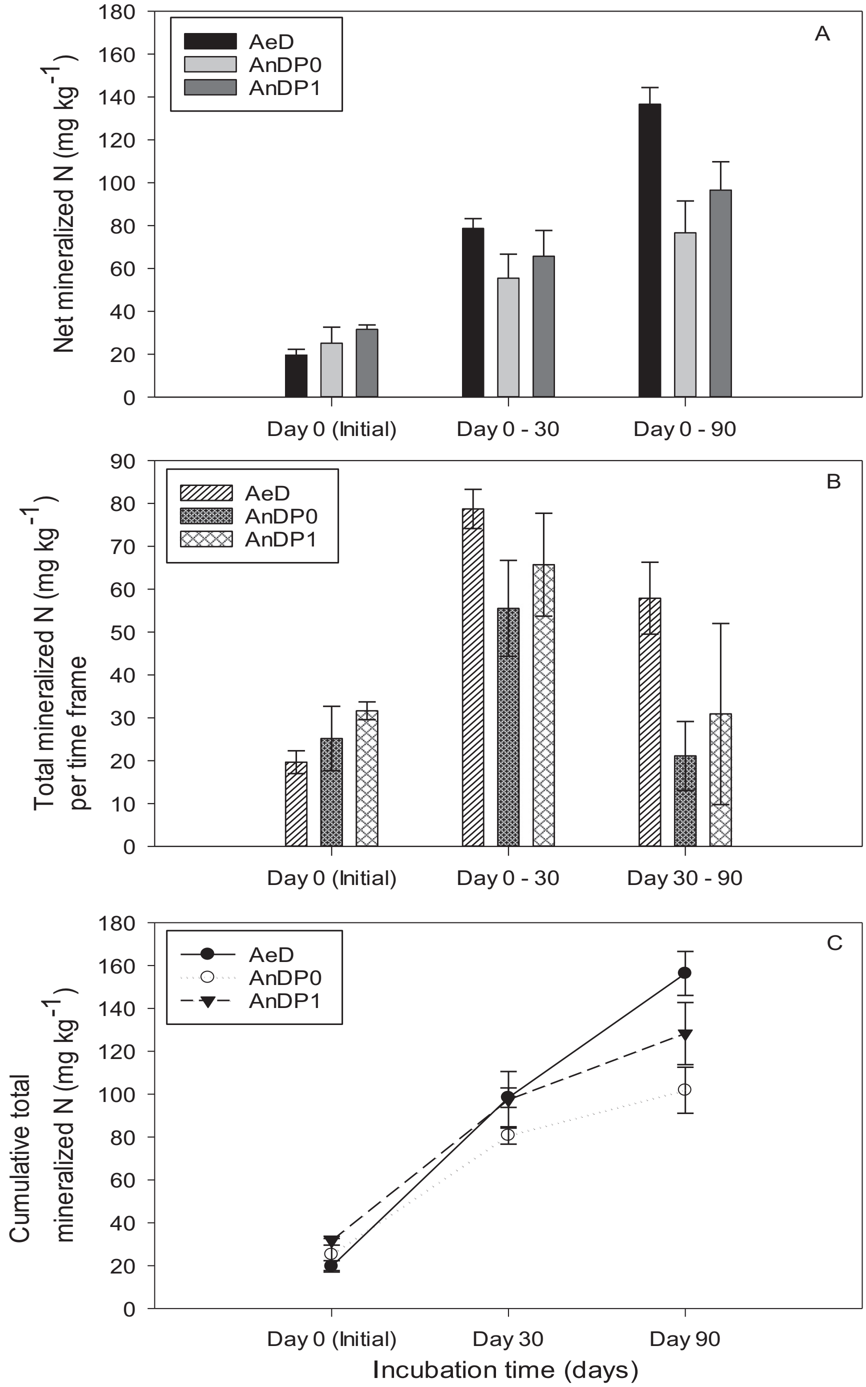

Fig. 5. Net mineralized N across the period (A), total mineralized N per incubation phase (B) and cumulative total mineralized N (C) from aerobic (AeD) and anaerobic without polymer (AnDP0) and anaerobic with polymer (AnDP1) sludges. Error bars represent standard deviation from the mean of three replications per treatment.

and a wide range of N mineralization rate from soils amended with biosolids (Rouch et al., 2011; Rigby et al., 2016; Manirakiza et al., 2019; Hseu and Huang, 2005; Rojas-Oropeza et al., 2010). The wide variation observed from such studies was mainly attributed to several factors that influence N mineralization with C:N ratio, stabilization process and the organic amendment type being the major ones.

There was a decrease in N mineralization rate for all sludges between Day 30 and Day 90. This is evident that OM stability was a great factor controlling mineralization. In this case, easily mineralizable material was almost exhausted by Day 30 leaving much of the hard to decompose material which probably required more time for microbes to decompose hence the decrease in NMR percentage was observed in the last 60 days of incubation. Generally, mineralization rate observed in the last 60 days was overall decreasing compared to the rate recorded within the first 30 days of incubation. This is an indication of easily mineralizable materials being gradually exhausted with time during the incubation period.

The amount of mineralized N is basically a function of applied organic N and the mineralization rate among other factors. Comparatively, AeD had the lowest N release rate per organic N applied, however, its cumulative mineral N over time remained higher than AnD sludges. The higher amount of mineralized N observed from AeD is attributed to the higher fraction of organic N as well as total N per unit mass of sludge applied. Applied organic N from AeD was higher than AnD sludges (Fig. 2) and by Day 30, about

79 mg per total applied organic N was mineralized (Fig. 5) and this accounted for 23.8% of organic N which would translate to 238 g mineral N kg^{-1} organic N applied. Similarly, for AnD sludges, about 56 and 66 mg were mineralized from total applied organic N and accounted for 31.5% and 36.7% translating to 315 g and 367 g mineral N kg^{-1} organic N applied for AnDP0 and AnDP1 sludges respectively. Thus, the results show that in this study the well stabilized AnD sludges released mineral N at equivalent or faster rates than unstabilized AeD sludge. This may have been influenced by air drying as noted above.

3.6. Net mineralized N and total mineral N supply from sludges

Mineralized N released during each incubation time and its subsequent cumulative totals are presented on Figs. 5A – C. Nitrogen fertilizer value of biosolids is a function of its mineralizable N fraction of the organic N. A significant variation in mineralized N was observed between sludges and it was influenced by sludge types at any moment during the incubation period. Within the first 30 days of incubation, all the sludges released different magnitudes of mineral N (Fig. 5A). There were substantial magnitudes of mineralized N within the first 30 days of incubation compared with the last 60 days, which show that mineralization process occurrence was faster in earlier days of biosolids application (Fig. 5B). The second phase of mineralization after 30 days showed a reduced N turnover rate during incubation (Fig. 5B). The reduced mineralization magnitude in the last phase resulted in a significantly lower mineralized N relative to the first phase although the last phase was a longer period (60 days) of organic N exposure to driving factors of mineralization.

This implies that when planning on producing crops fertilized with biosolids, for maximum utilization of the mineralized N from such materials, the timing of application should be strategically planned so as to synchronize the period on maximum N release with critical crop growth stages of high N requirements. This is an important for environmental protection strategy since mineralized N like nitrates are prone to leaching if exposed to possible driving factors in the absence of crops. Therefore, synchronizing crop growth stages of critical N needs with the period of high N mineralization (Tao et al., 2018) may reduce chances of N losses for example, through leaching since most of it would be taken up by crops. The pattern of cumulative total mineralized N (Fig. 5C) followed the nitrification trend (Fig. 3A). This shows that the total sludge mineral N released during the study was dominated by the $NO_3 – N$ fraction, as could be observed on Fig. 3B where the nitrification process was positive relative to the net negative ammonification (Fig. 3A).

Based on the final NMR% (at Day 90), sludge TN%, and the calculated sludge organic N of about 94%, 88% and 85% of the applied sludge total N for AeD, AnDP0 and AnDP1 respectively (i.e TN applied less mineral N applied in soil-sludge mix) at a sludge rate of 10 tons ha^{-1} $season^{-1}$, mineral N (fertilizer value from sludges) would have been applied in the order of AeD > AnDP1 > AnDP0. By the end of the incubation study, AeD sludge would have supplied through mineralization approximately 268 kg mineral N per total organic N applied ha^{-1} $season^{-1}$. This is almost ± 1.5 times higher than the final mineral N supply from AnD sludges of which the later would supply about 168 and 191 kg mineral N per total applied organic N ha^{-1} $season^{-1}$ from AnDP0 and AnDP1 respectively. This implies that AnDP1 would eventually add ~23 kg mineral N ha^{-1} $season^{-1}$ over and above the supply from AnDP0. Cumulatively, AeD sludge type remained superior in the size of mineralized N over AnD sludges; this can be attributed to the amount of applied organic N at the beginning of the study.

It is interesting to note that, the observed laboratory mineralization rate especially for AnDP1 concurs with N release from some field incubation study across agroecological zones (Badza et al., 2020c). The final N mineralization rate of around 54% was within the range of the nine months N mineralization recorded from high rainfall receiving areas which ranged between 48 and 57% in field study. This brings up some important messages regarding reliability of laboratory studies results and their use in driving real field sludge application. This implies that when there is lack of field studies, laboratory outcomes can be used to a certain extent, in this case, it will be reliable to base sludge application rate on laboratory studies for high rainfall areas, however, the same cannot be applicable to arid and semi-arid regions.

For N management purposes and to minimize excessive N application into the environment, a uniform application rate across sludges of different types or sludges that went through different treatment processes is not a feasible and sustainable option and might be misleading in decision making towards sludge N management. Addition of polymer may influence mineral N release, although further research is needed to verify this. Approximately an additional 23 kg mineral N ha^{-1} $season^{-1}$ could be released from anaerobic sludge treated with polymeric material. Therefore, the findings strongly suggest and affirm for proper characterization and optimization of individual sludge application rates largely based on nutrient content rather than a "blanket" rate to enable sustainable agroecosystems and limited N pollution that could be possible through leaching of excess nitrates.

4. Conclusions

Understanding N mineralization is key in designing sustainable N management strategies from biosolids applied in agricultural lands that would limit environmental pollution from excess nutrient application. Cumulative N release is a function of initially applied organic N and N mineralization rate. The study findings revealed that the magnitude of mineralization is greater in the early days of biosolids application. Generally, C and N mineralization were influenced by sludge stabilization processes. Sludge that have undergone through aerobic treatment are likely to release high mg $CO_2 – C\ kg^{-1}$ soil relative to anaerobic sludges. Anaerobically digested sludges applied to soil showed an equal or higher net N mineralization rate than aerobic sludge. Overall, by the end of the study, higher N supply was realized from aerobic than anaerobic sludge, although the N mineralization rate was lower. This was because the aerobic sludge had substantially greater TN than the AnD materials. This affirms that sludge applications should be based on nutrient content (TN and mineralizable organic N) and mineralization rate rather than a single and generalized recommendation rate. As such, this strategy would limit excess application of nutrients hence reducing pollution whilst enriching agroecosystems.

For future research, these biosolids materials should be tested in field experiments to establish N release rate under field conditions and how they relate to laboratory findings. Further research is required on potential synchronization timelines of high mineralization rate from biosolids and crop growth stage for maximum plant nutrient uptake. It could be also insightful for future work to investigate principles of how polymer addition might affect nutrient availability in biosolids.

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Declaration of Competing Interest

The authors declare no conflict of interests.

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